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1. Your reference — GM/99058 GB

2. Patent application number
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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

MEDEX SCIENTIFIC (UK) LIMITED
THE OLD GRAIN STORE,
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Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — UNITED KINGDOM

4. Title of the invention

PLANT EXTRACT MIXTURES AND THEIR USES

5. Name of your agent *(If you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

BATCHELLOR, KIRK & CO.,
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Patents ADP number *(if you know it)* 31500 1

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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PLANT EXTRACT MIXTURES AND THEIR USES

This invention is concerned with mixtures comprising plant extracts from plants in three families (one or more of Cissus, Vernonia and Brillantasia), and uses of the mixtures to enhance fat-binding capacity as well as the inhibition of carohydrate breakdown, amylase activity and nutrient absorbtion in the presence of fat binding materials. In particular the mixtures can be combined with chitosan or chitosan derivatives to synergise their fat retaining qualities.

Fat binding materials e.g. chitosan have applications in industry and in health. In both cases, the binding capacity of these materials is limited because of their bulk. The new combination of plant extracts has the ability to enhance the fat binding capacity of such materials as well as alter the metabolism of other compounds in animals, including humans.

According to one aspect of the present invention there is provided a composition comprising extract of one or more plants of one or more of the following plant families: Cissus, Vernonia and Brillantasia. According to another aspect the invention provides uses of such compositions in treating disease or disorder. In another aspect the invention provides such compositions for the preparation of a medicament for use in treating any of the ailments herein described.

The composition preferably essentially consists of a mixture of at least two of the said plant extracts. The composition may also include one or more fat binding materials such as chitosan or a chitosan derivative, one or more amylase inhibiting compounds and/or one or more antioxidants such as vitamins A, C, or E

The extract may be of the leaves, roots and/or stem of the plants.

water and boiled for 1 hour. The mixture is left to stand for 2 hours at room temperature before straining to remove any residue. The resultant supernatant is stored at 4°C until required.

Brillantasia sp.

Plant component used: Leaves

Preparation method: The leaves are harvested from the plant and dried at 45°C for 72 hours. The dried leaves are ground into a powder, then transferred into 2 times their weight of boiling water. This is left to stand for two hours at room temperature before straining to remove leaf residue. The resultant supernatant was stored at 4°C until required.

Cissus sp. and Vernonia sp. combination

Although all combinations of extracts of plants of the three families, namely Cissus sp. Vernonia sp. and Brillantasia sp. had an enhancing effect, optimal activity was obtained using the following mixture of two aqueous plant extracts:

Reduction of fat absorbed by the body

Increases in the amount of fat in faeces

Increase of faecal bulk

Reduction of carbohydrate breakdown in vivo

Decrease in acidity of the stomach

Increase in the amount of cholesterol in the faeces

Reduction of post-prandial blood glucose

Reduction of Body mass index (BMI) and weight

The mixture can be taken orally at a concentration of 0.1 to 10ml, preferably 0.1 to 5ml, more preferably 0.2 to 2ml, most preferably 0.5ml per kilogram body weight with or before a meal.

Inhibition of salivary and pancreatic amylase activity

Human salivary amylase (Sigma A 0521) and porcine pancreatic amylase (Sigma A3176) were used as starting material. The substrate used was starch and the formation of maltose was used to quantify and measure the activity of the amylase. One unit of activity of the mixture reduced the activity of salivary amylase by 50%, and the activity of pancreatic amylase by 65%.

Decrease of acidity of the stomach Laboratory animals were fed diets containing the mixture (0.5ml/kg body weight) after an overnight fast. The content of their stomachs had a higher pH than control animals.

Humans who ingested the mixture produced faeces with a lower pH than control humans who had not ingested the mixture.

Inhibition of pancreatic lipase activity

Pancreatic lipase (Sigma L9780) was used as starting material.

The effect of chitosan based formulations on fecal weight, pH, ash, calcium, magnesium and total lipid content in overweight women

Abstract

Chitosan is widely used for the control of weight. Its use is based on its ability to bind fatty acids *in vitro* and *in vivo*, thereby reducing the ability of the body to absorb and utilise dietary fats. By combining chitosan with two plant extracts; Cissus *quandragularis* and Vernonia *glabra*, its ability to bind fatty acids and triacylglycerols *in vitro* was significantly increased ($p<0.05$ and $p<0.01$ respectively). This is reflected in the significantly higher fat content of the faeces of subjects on chitosan or chitosan based formulations. Compared to chitosan, the combination of chitosan and Cissus *quadrangularis* significantly increased the fecal pH ($p<0.01$), fecal ash ($p<0.02$) and mineral content ($p<0.02$) in overweight women (BMI 25-29 kg/m^2) over a six month period, while decreasing the fecal water content ($p<0.01$). The combination of chitosan and Vernonia *glabra* did not alter the mineral content or the pH of the faeces although it caused a significant increase ($p<0.05$) in fecal bulk. The results indicate that the chitosan and Vernonia *glabra* combination can be effectively used in long term weight control regimes.

Key words. **Overweight , Obese , Fecal fat, Fecal minerals, Chitosan, Plant extracts , Ash**

INTRODUCTION

Chitosan is a polysaccharide produced from chitin found in the exoskeletons of arthropods (crustaceans and insects) and the endoskeletons of cephalopods. It is widely spread in the biomass, being the most abundant biopolymer after cellulose (Furda, 1983; Roberts, 1992). It is generally accepted that chitin is extensively acetylated while chitosan is largely deacetylated (Furda, 1983). Chitosan is a cationic glucosamine polymer with a high anion-exchange capacity as a result of quaternary ammonium ions. It is known to have a marked hypocholesterolemic effect in rats (Sugano *et al* ,1978), alters bile acid metabolism (Yasuhiko et al ,1991) and increases HDL:total cholesterol ratio in broiler chickens (Razdan et al ,1993). The hypocholesterolemic effect of chitosan can be theoretically explained by its ability to decrease lipid absorption and increase fecal cholesterol excretion. The alteration of bile acid can be due to the modification of colon pH (Yasuhiko *et al* ,1991)

This study investigated the effect of chitosan and chitosan based formulations on overweight women. Their BMI and body composition had been studied in related

Diet

The subjects were asked not to change their food habits and to maintain their normal diets as much as possible. They kept individual food diaries which were used to analyze food intake using food tables.

Fecal collections and analysis .

Faeces were collected for four consecutive days every other week, in special glass containers and brought daily to the laboratory for storage. All subjects were instructed to bring in their samples as soon as was possible. The feces was weighed and the pH measured. An aliquot (2 g) of the faeces was used for the determination of total lipid using the method described by Folch *et al,* (1957). The dry matter weight was determined using a homogenate of the total feces collected (3 days drying at 55°C).
Fecal ash was determined after 48 hours incineration at 500°C. Fecal calcium and magnesium was determined using the modified methods described by SIGMA company. In these methods, the ash was dissolved in nitric acid solution (3N) and the calcium and magnesium assayed spectrophotometrically using arsenazo dye III and calmagite respectively. Total nitrogen was determined by the Kjeldahl procedure.

Statistical significance was determined using paired Student's t-test.

Results and discussion

In vitro lipid binding

The *in-vitro* lipid binding of the different chitosan formulations is shown Table 2. The highest lipid binding was obtained with the formulation CF_1 for each of the two plant powders containing 16% (w/w) of the plant. Cissus *quandragularis* however bound more *($P<0.001$)* lipid than *Vernonia glabra ($P<0.01$)*. Other combinations containing Cissus quadrangularis enhanced the binding of soya oil. The binding was however not as high as for the CF1 combination containing 16%(w/w) plant powder.

Table 2. *The effect of Chitosan formulations on in-vitro lipid (Soja oil) binding (g g of Chitosan formulation). mean±S.D*

Plant Powder	Chitosan formulations				
	CF_0	CF_1	CF_2	CF_3	CF_4
V.glabra	13.70±2.04	22.04±2.79**	17.81±4.09	12.49±4.78	10.44±4.9
C.quandragula	13.70±2.04	27.80±0.47**	22.60±6.52**	18.03±1.17*	14.89±2.05

*P<0.01 **P<0.001

This study has shown a negative correlation between the fecal moisture and the fecal pH (r=-0.477) and even between fecal moisture and the root square of fecal pH [$(pH)^{1/2}$] (r=-0.465), P<0.001. So the reduction of fecal moisture can be explained by the increase of the fecal pH.

The reduction of fecal moisture is also dependent on the ash content since fecal moisture is negatively correlated to the fecal ash content (mg/g of FDW) (r=-0.301), P<0.01 (Table 5)

Table 5: *Correlation between different fecal components*

Variable	r (N=80)	Probability(P)
♦ moisture, pH	-0.477	P<0.001
♦ moisture, $(pH)^{1/2}$	-0.465	P<0.001
♦ moisture, ash	-0.301	P<0.01
♦ ash ,pH	+0.248	P<0.05
♦ calcium, pH	-0.221	P<0.05
♦ moisture ,calcium	+0.030	N.S
♦ ash , calcium	-0.069	N.S
♦ pH ,Nitrogen	-0.232	P<0.05
♦ ash , Nitrogen	+0.009	N.S
♦ moisture , Nitrogen	+0.011	N.S

Chitosan as well as the chitosan formulation containing *Cissus quadrangularis* significantly ($p<0.05$) increased the fecal calcium concentration after one month of treatment (Table 6). The formulation containing *Vernonia glabra* however brought about a decrease in the concentration of fecal calcium. It is likely that the increased concentration of calcium in feces is as a result of contribution from the chitosan. There however seems to be a factor in the formulation containing *Vernonia glabra* that causes an increased retention of calcium.

Unlike calcium, magnesium concentrations in the faeces was not altered by any of the formulations (Table 6).

Fermentation of chitosan in the large intestine produces glucosamine which can be absorbed (application in arthritis) by the body. Glucosamine can also bring about an increase in gut pH which favors the absorption of nitrogenous compounds (negative correlation between fecal pH and total fecal nitrogen, $r = -0.232$; $p<0.05$). The formulation containing *Vernonia glabra* however significantly ($p < 0.05$) increased the amount of nitrogen present in the faeces (Table 6). This increase is still significant even when the amounts contributed by the presence of chitosan is taken into consideration. On the other hand, fecal nitrogen is significantly ($p < 0.05$) reduced by the chitosan formulation containing *Cissus quadrangularis*.

Vernonia glabra therefore seems to play a role in inhibiting the absorption of nitrogenous compounds which will otherwise be favored by an increase in pH.

Chitosan as well as chitosan based formulations significantly ($p<0.01$) increased the total amount of lipid in the faeces (Table 7). This is as a result of the ability of chitosan to bind lipids in the gut. (It will however be interesting to investigate what happens to the mixture of lipids, chitosan and glucosamine in the large intestine).

CONCLUSION

The results of this study show that the chitosan only increase the fecal pH and cause the constipation while CF1.V decrease the fecal pH. These results suggest that the long term intake of chitosan can cause colon cancer while the use of CF1.V can be benefit for overweight and obese patients.

Table 4: *The effect of Chitosan formulations on fecal wet and dry weight , pH and moisture*

Measures	Control (n=20)		Chitosan (n=21)		Chitosan+V.g(n=18)		Chitosan+C.g(n=21)	
	D_0	$D_{>30}$	D_0	$D_{>30}$	D_0	$D_{>30}$	D_0	$D_{>30}$
Fecal wet weight(g/d)	165.4±26.4[a]	187.8±41.5[a]	185.7±24.6[a]	180.2±16.7[a]	178.5±39.0[a]	264,2±33.[b]	188.1±28.6[a]	187.8±82.[a]
Fecal dry weight(g/d)	26.3±5.3[a]	28.5±9.8[a]	29.0±5.3[a]	28.9±9.7[a]	28.2±8.2[a]	36.0±9.4[b]	28.2±7.7[a]	27.2±9.4[a]
Fecal moisture %	79.8±4.9[a]	80.1±5.2[a]	78.6±5.9[a]	72.6±6.7[b]	79.1±4.0[a]	81.0±4.5[a]	78.9±3.8[a]	74.9±6.1[ab]
Fecal pH	6.9±0.6[a]	6.8±0.8[ac]	6.9±0.4[a]	7.4±0.3[b]	6.8±0.2[a]	6.5±0.3[c]	6.9±0.5[a]	7.0±0.3[a]

Values are 4-days means±S.E. Values without a common superscript are significantly different ($P<0.05$)

Table 7: *The effect of chitosan formulations on total lipid excretion*

	Control (n=20)		Chitosan(n=21)		Chitosan+V.g(n=18)		Chitosan+C.q(n=21)	
	D_0	$D_{>30}$	D_0	$D_{>30}$	D_0	$D_{>30}$	D_0	$D_{>30}$
Total lipid(mg/g)[a] g/d	73±15[a]	87±15[a]	83±16[a]	163±18[b]	86±19[a]	183±12[b]	88±16[a]	161±6[b]
	8.4±3.7[a]	10.0±4.0[a]	9.6±4.1[a]	29.4±14.2[b]	9.9±4.5[a]	48.3±9.2[b]	10.2±3.9[a]	30.3±13.2[b]

Values are 4-days means±S.E Values without a common superscript are significantly different ($P<0.01$)

Diet

The subjects were given a number of possible diets they could follow, which provided a total daily energy intake of 1500 kcal.

Body mass index (BMI) and Body fat content

The BMI of subjects was measured using an electronic scale and a metre rule attached to the wall. The percentage body fat was determined by using bioelectrical impedance measurements.

Blood collection and sampling

Venous blood (20 ml) was collected from the forearm of subjects, and serum prepared from it was stored in 1ml vials at -70°C until required.
The concentration of total cholesterol and triglycerides were determined using Sigma kits.

Results

Body Mass Index (kg/m^2). *Values are means± sem.*

	D_0	D_7	D_{15}	D_{30}	D_{60}	D_{90}	D_{120}	D_{150}	D_{180}	D_{210}
Control	30.61± 2.03	31.61± 2.03	29.41± 2.42	29.21± 1.68	29.03± 1.76	29.03± 2.34	30.04± 1.67	29.87±1 .98	29.22± 2.10	28.69± 3.01
Chitosan	28.66± 1.78	28.60± 1.78	27.31± 2.02	26.02± 2.01	26.03± 1.67	25.34± 1.67	27.87± 1.87	28.02±1 .67	26.21± 2.02	26.04± 1.53*
Chitosan + Vernonia glabra	29.92± 2.17	29.83± 2.18	28.33± 1.67	26.18± 1.78	25.87± 1.23*	25.34± 1.56*	25.02± 1.78*	24.98±1 .82*	25.38± 1.56*	24.88± 1.67*
Chitosan + cissus quadrangularis	28.43± 1.53	28.62± 1.33	28.04± 1.89	26.44± 0.98	24.64± 1.22**	23.40± 2.68**	23.55± 2.67**	25.60±3 .56	24.36± 1.78*	24.31± 1.50*

*$p \leq 0.05$; **$p \leq 0.01$

Conclusions:

Subjects on a daily average energy intake of approximately 1500 kcal, did not show any significant change in BMI. Subjects on the formulations containing *Vernonia glabra* as well as *Cissus quadrangularis* had reduced BMIs after being on the formulation for 60 days.

Total Blood Triglycerides (g/L)

	Day 0	Day 7	Day 15	Day 30	day 60
Control	1.84 ± 0.08	1.83 ± 0.08	1.76 ± 0.10	1.89 ± 0.09	1.76 ± 0.18
Chitosan	1.26 ± 0.13	1.18 ± 0.05	0.65 ± 0.14**	0.83 ± 0.11*	0.74 ± 0.10**
Chitosan + vernonia glabra	1.87 ± 0.31	1.19 ± 0.08*	0.82 ± 0.09**	0.73 ± 0.06**	0.68 ± 0.08**
Chitosan + Cissus quadrangularis	1.84 ± 0.32	1.49 ± 0.27	0.62 ± 0.14**	0.84 ± 0.07**	0.70 ± 0.10**

*$p \leq 0.05$, **$p \leq 0.01$. *Significant differences are by comparing to the control.*

Conclusion:
Chitosan and chitosan formulations significantly decreased the circulating concentrations of triglycerides. This is as a result of their ability to bind triglycerides *in vitro* as well as *in vivo*. The presence of plant extracts did not seem to have a potentiating effect on the ability of chitosan to bind triglycerides.

Part 2.

The effect of *Cissus quadrangularis* and *Vernonia glabra* combination on blood lipid levels

The mixture used in this part of the work had the following composition:

Chitosan	61.8%
Vitamin C	19.0%
Vernonia glabra powder	5.7%
Cissus quadrangularis powder	13.5%

This mixture had a superior lipid binding capacity *in vitro* compared to other chitosan formulations containing either Vernonia glabra or Cissus quadrangularis.

In Vitro Lipid binding capacity of mixture

32.6 grams oleic acid per gram of mixture
(independently verified by Professor Alain Domard; note attached).

Effect of mixture on BMI in overweight adults (BMI > 25)

Body Mass Index (kg/m^2).

Values are means± sem.

	D_0	D_7	D_{15}	D_{30}	D_{60}	D_{90}	D_{120}	D_{150}	D_{180}	D_{210}
Control	30.61± 2.03	31.61± 2.03	29.41± 2.42	29.21± 1.68	29.03± 1.76	29.03± 2.34	30.04± 1.67	29.87±1 .98	29.22± 2.10	28.69± 3.01
Chitosan	28.66± 1.78	28.60± 1.78	27.31± 2.02	27.02± 2.01	26.03± 1.67	28.04± 1.67	27.87± 1.87	28.02±1 .67	26.21± 2.02	26.04± 1.53*
Chitosan +	29.92±	29.83±	28.33±	26.18±	25.87±	25.34±	25.02±	24.98±1	25.38±	24.88±